

Appl. No. 10/019,586
Supp. Amdt. dated June 16, 2004

Patent Docket No. P1746R1

Amendments to the Specification

Please replace the paragraph beginning at page 24, line 35 with the following paragraph:

DHFR, the desired protein and GFP can be expressed from one promoter to improve the co-expression efficiency. For example, GFP and DHFR can be expressed as a fusion protein, or an IRES can obviate the need for a second promoter to express GFP. In the constructs shown in Figure [[9]]], rows 1 and 2, the exemplary amplifiable selectable gene, DHFR, is fused to the GFP gene to form a DHFR-GFP fusion gene. Each of the upstream and downstream coding sequences (in the first example in Figure [[9]]], row 1, the upstream coding sequence is DHFR-GFP fusion gene; in the second example represented in row 2, the upstream coding sequence is the selected sequence) has its translational stop signal. Translation initiates again for the downstream coding sequence. These scenarios allow expression of two separate proteins from a single promoter. It will be understood that the positioning of the promoter/enhancer, translational stop signal, translational initiation site, transcription termination site and polyA signal, relative to the various components in each transcription unit, as described here, apply to all the constructs described below.

Please replace the paragraph beginning at page 27, line 10 with the following paragraph:

The constructs of the invention can also comprise two expression/transcription units, as shown in Figure [[9]]], rows 4-9. The two-transcription unit construct depicted in Figure [[9]]], row 4, comprises one selected sequence. Rows 5-9 show constructs wherein two selected sequences can be inserted, one in each transcription unit. Each of the two transcription units will comprise a promoter and optionally, an enhancer, a transcriptional termination site and polyA signal sequence. The second transcription unit can use the same or different kind of promoter as used in first transcription unit. For example, both transcription units can use the SV40 promoter. One or both of the transcription units can comprise an intron.

Please replace the paragraph beginning at page 27, line 17 with the following paragraph:

Figure [[9]]], row 4, illustrates a construct wherein the first transcription unit contains DHFR in an intron (the first intron), followed by the selected sequence. The second transcription unit will comprise the GFP gene. The second transcription unit will preferably comprise an intron (referred to as the second

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intron) immediately 5' of the GFP. The three coding sequences are still physically linked in one vector but are independently transcribed from two promoters. The primary transcript produced from the first transcription unit encodes both DHFR and the selected sequence but only the DHFR gene is translated into product. Preferably, at least 95% of the transcripts will have the DHFR gene spliced out and will translate into the desired product. In the second transcription unit, if the GFP is placed downstream of an intron, both spliced and unspliced transcripts from this transcription unit will produce GFP.

Please replace the paragraph beginning at page 27, line 33 with the following paragraph:

In yet another embodiment of the preceding construct comprising two transcription units and two introns, instead of placing the GFP gene within the second intron in the second transcription unit, an IRES is placed between the second selected sequence and the GFP gene (Fig. [[9]]L, row 6). Both the second selected sequence and the GFP gene from the second transcription unit will be translated from the dicistronic message.

Please replace the paragraph beginning at page 28, line 3 with the following paragraph:

In still another variation of the construct comprising two-transcription units and two introns, the first intron in the first transcription unit is left empty but an IRES is inserted downstream of the first gene of interest to allow translation of a downstream DHFR-GFP fusion gene. The second transcription unit will comprise the second intron followed by a second gene of interest (Fig. [[9]]L, row 8). Optionally, another selectable marker gene (other than the amplifiable selectable gene and GFP gene), can be placed within the second intron or the intron can remain without an inserted gene.